To control proliferation and apoptosis, B-non-Hodgkin lymphomas utilize a plasma membrane raft-associated signalsome made of the constitutively active Lyn kinase, the tyrosine phosphorylated Cbp/PAG adaptor and tyrosine phosphorylated STAT3 transcription factor. No such signalsome is found in rafts of ALK+ T-lymphoma and Hodgkin-derived cell lines, despite similar Cbp/PAG, Lyn and STAT3 expression, and similar amounts of raft sphingolipids. We suggest that stable association of the signalsome with B-NHL rafts requires (i) a Lyn kinase (auto)phosphorylated in its regulatory and active site tyrosines, (ii) a Cbp/PAG adaptor phosphorylated at tyrosine 317 and bound to Lyn SY2 via phosphotyrosine 299 and neighboring residues and (iii) a tyrosine phosphorylated STAT3 linked via SH2 to the regulatory, C-terminal tyrosine of Lyn. The cytoplasmic Csk kinase that negatively controls the Src kinase activity in human T lymphocytes is not involved in the B-NHL signalsome, strongly suggesting that associated Lyn and Cbp/PAG form a constitutively active signalsome in B-NHLs. An oncogenic role for Lyn was shown following exposure of B-NHL cell lines to small molecular weight Lyn inhibitors that prevented Lyn and Cbp/PAG phosphorylation, dissociated the signalsome from rafts and eventually induced apoptosis. Apoptotic cell death also followed decreases in Lyn or Cbp/PAG expression levels in one mantle-cell lymphoma line, but not in a Hodgkin-derived one, supporting the notion that B-NHLs are oncogenically “addicted” to the Lyn/PAG signalsome. The Lyn-Cbp/PAG signalsome therefore exerts a proximal control on proliferation and survival in most B-NHLs, and represent a suitable therapeutic target in B-NHL cells.

Results: TW-37 inhibited cell growth and induced apoptosis on all established cell lines and fresh tumors with IC50 ranging between 165 nM and 320 nM. The compound binds with highest affinity to Bcl-w (1.48 nM) and lowest affinity to Bcl-2 (150-170 nM); equilibrium binding to Mcl-1 occurs at 27 nM. TW-37 competes with Ibad for the binding pocket of Bcl-2. However, it was not able to disrupt an already formed Bcl-2-tBid heterodimer with TW-37 concentrations as high as 10,000 nM. WB analysis of all B2 family proteins suggested that TW-37 response is best predicted by Bax/A1 ratio which varied over 6-folds between the 4 cell lines tested. So far, fresh patient samples fit this curve.

Conclusions: SMI of B2 family proteins are effective in inducing growth inhibition and apoptosis of lymphoma cells. Such molecules must bind to their targets before heterodimers form between natural pro- and anti-apoptotic proteins. A high Bax/Mcl-1 ratio in lymphoma cells may be an important predictor of response to TW-37. This compound may stand apart from other BH3 mimetic SMIs in its ability to hit all drug targets including Mcl-1.
Hematopoietic tumors are caused by the activation of oncosenes and inactivation of tumor suppressor genes. Previous reports have shown that using conditional transgenic model of MYC-induced lymphoma, inactivation of the MYC oncogene can result in sustained regression of hematopoietic tumors. Recently, we have found that the HMG-CoA reductase inhibitor, atorvastatin has potential clinical activity in patients with lymphoma. To measure the influence of this and other agents on the regulation of oncosenes and tumor suppressors and associated signaling transduction pathways in patients, we have developed a novel nano-scale immunassay system to measure changes in expression and activation of proteins including MYC, ERK, STAT3 and STAT5 in pre-clinical mouse models as well as human patients with lymphoma and CML before and after therapeutic oncogene inactivation treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment. We will present data illustrating that atorvastatin surprisingly exhibited proteomic changes in fine needle aspirates from patients before and after therapeutic treatment.

**392 THE ROLE AND PROGNOSTIC SIGNIFICANCE OF Ki-67 INDEX IN NON-HODGKIN’S LYMPHOMA**

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Introduction: An important and easy to use proliferation marker is the nuclear protein Ki-67, which is an antigen present in all proliferating cells and thus determines the growth fraction of tumors.

Material and methods: The Ki-67 proliferation index (PI) assayed immunohistochemically in tissue samples of 319 newly diagnosed NHL patients was evaluated according to the WHO lymphoma classification and NCI grade. In 268 patients the correlation between Ki-67 PI and the clinical course and survival outcome was also analyzed.

Results: There was a significant difference in mean Ki-67 PI among different lymphoma sub classifications (p<0.001). The mean Ki-67 PI in indolent lymphomas was 26.6%, in aggressive lymphomas 67.2% and in highly aggressive lymphomas 97.6% (p<0.001). A cut-off value of 45% was assessed by ROC curve (area under the curve = 0.877, p<0.001) as differentiating between indolent and aggressive lymphomas. It detected 82.8% of the indolent lymphomas (Ki-67 PI <45%) and 85% of the aggressive lymphomas (Ki-67 PI >45%). In patients with DLBCL (n=141), a correlation was found between low or high Ki-67 PI (cutoff at 70%) and patient age and performance status (PS). A high Ki-67 PI was found in 41.9% of younger patients (age <60) compared to 61.4% of older patients (age >60) (p=0.025). A high Ki-67 PI (>70%) was found in 49% of patients with good PS (0-1) compared to 69% of patients with poor PS (PS 2 and higher) (p=0.04). The 3 years survival of DLBCL patients with low Ki-67 PI was 75±4.6% compared with 55±9.6% in patients with high Ki-67 PI (p=0.015). When combining the Ki-67 PI with other prognostic factors, like the IPI score, and disease bulk, Ki-67 PI added to prognosis evaluation in the group of patients
with low IPI (52) and patients with disease bulk >10cm. The 3 years survival in patients with low IPI (52) was 94.6%, while if they had a low Ki-67 PI (<70%) and 64.8% if they had high Ki-67 PI (>70%), (p=0.01). Patients with bulky disease (>10cm) had a significantly better survival if they presented a lower Ki-67 PI value (3% survival of 100% vs. 25%, p<0.02).

Conclusions: Different lymphoma entities have different Ki-67 index. A cutoff value of 45% can differentiate between indolent and aggressive lymphomas. In DLBCL a cutoff of 70% differentiated between good and bad prognosis and correlated with patient age and PS. Ki-67 PI adds to the prognosis of DLBCL patients with low IPI and patients with bulky disease.

400 IGVH GENE MUTATION STATUS AND GENOMIC IMBALANCES IN CHRONIC LYMPHOCYTIC LEUKAEMIA WITH INCREASED PROLYMPHOCYTES (CLL/PL)

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Chronic lymphocytic leukaemia (CLL) with increased prolymphocytes (CLL/PL) has been defined by the World Health Organisation (WHO) classification and considered as a progressive and clinically aggressive variant of CLL. To further characterize the biological features of this disease, we performed IGVH gene mutational status, FISH and high resolution comparative genomics hybridization (HR-CGH) analysis in 17 cases of CLL/PL. ALL CLL/PL utilized members of V H1, VH3 and VH4 families, with the highest prevalence of the VH1-69 gene. In all but one case analysed, the VH1 gene was unmapped. The FISH and HR-CGH analyses showed frequent occurrence of trisomy 12, del(11)(q23), del(17)(p13) and genetic imbalances, but recurrent genetic lesion characteristic for CLL/PL was not found. The follow-up HR-CGH analysis of two cases showed that increase of prolymphocytes in the course of CLL or CLL/PL is associated with clonal evolution and selection of the tumour clone. In conclusion, this study suggests that CLL/PL is a relatively homogeneous disease regarding V H gene mutation, but heterogeneous regarding genetic lesions. The heterogeneity and high number of genetic imbalances found in CLL/PL suggest that a genome-wide instability of the neoplastic cells may play a role in the development of the disease.

401 NK-S1, AN NK/T NON-HODGKIN’S LYMPHOMA XENOGRAFT FOR DRUG TESTING AND THERAPEUTIC INTERVENTION

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NK/T non-Hodgkin’s lymphomas (NHL) are more frequently observed in Asia, South and Central America compared with the United States and other Western populations. Disseminated NK/T NHL is mostly incurable and responds poorly to conventional anthracycline-based regimens that are used to treat B cell NHLs. We have established an EBV positive NK lymphoma xenograft (NK-S1) in SCID mice, from the testicular metastasis of a patient who presented with nasal-type NK lymphoma. The xenograft retained the same immunohistologic features of the original, and was positive for cytotoxic CD3, TIA-1, granzyme B and EBER. This xenograft has been passaged exclusively in vivo. We have previously tested and found doxorubicin to have no effect on the growth of subcutaneous NK-S1 xenografts. In this study, we found that both IP ganciclovir (600mg/kg q2d x7 and q2/3d) and IV oxaliplatin (q7d x3) caused complete remission of the tumors. However at D39 after treatment was first instituted, tumour regrowth was observed. IP campath (200mg/dose q3d x10) also caused tumors to go into complete remission of the tumors. However at D39 after treatment was first instituted, tumour regrowth was observed. IP campath (200mg/dose q3d x10) also caused tumors to go into complete remission of the tumors. However at D39 after treatment was first instituted, tumour regrowth was observed.
parallel, edelfosine has the same dose dependent effects on apoptosis (17 in measurements using DRBM as mitochondrial transmembrane potential indicator). Furthermore, chelation of intracellular Ca²⁺ ions with BAPTA-AM strongly reduced edelfosine-induced apoptosis. These data confirm the selective effect of edelfosine in a wide spectrum of cells and suggest the involvement of Ca²⁺ signaling in edelfosine-induced apoptosis. In addition, edelfosine augments the size of the Ca²⁺ mitochondrial store. This could result in the formation of permeability transient pores, the release of cytochrome c into the cytoplasm, and apoptosis. Due to its specific action on cancer cells, edelfosine is being used as an anticancer drug in myelomas, sarcomas, and prostate cancers. Our work shows that this ether lipid could also have a therapeutic interest in the treatment of B lymphoma, only or in association with chemotherapy.

403 EVIDENCE OF SGN-40-INDUCED CHEMOSENSITIZATION OF NON-HODGKIN’S LYMPHOMA TUMORS

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Background: SGN-40 is a humanized monoclonal antibody that targets CD40, triggers pro-apoptotic signal transduction, and mediates effector cell function (ADCC/ACDP). SGN-40 overcomes rituximab resistance and improves chemotherapy efficacy in xenograft models and has been shown to up-regulate the p55 family member TAP63α, a chemo-sensing transcription factor capable of inducing apoptosis when combined with cytotoxic agents.

Methods: Observational case series based on medical records review of patients (N=4) treated with gemcitabine immediately after progression on single-agent SGN-40. Patients received SGN-40 as participants on a clinical trial of SGN-40 in relapsed refractory NHL.

Results: Patient 1: 72 YO female with DLBCL, who previously failed multiple therapies (R-CHOP, ESHAP, lenalidomide, gemcitabine). The patient was enrolled on the SGN-40 study. After completion of Cycle 1 of SGN-40, progression was documented (largest tumor 12.3 x 6.8 cm left inguinal mass). Three days after the last dose of SGN-40, gemcitabine was started at a reduced dose due to thrombocytopenia (600 mg/m² on days 1, 8, and 15). After 2 months of therapy, CT scans demonstrated a PR (58% reduction) which lasted 7 months. Patient 2: 42 YO female with DLBCL failed multiple therapies (R-CHOP, RT, ASCT) with PR as best response. Following 1 cycle of SGN-40, progression was documented (largest tumor 11.3 x 5.1 cm periumbilical soft tissue mass). Two days after the last dose of SGN-40, gemcitabine was started (1000 mg/m² on days 1, 8, and 15). After 2 months of therapy, CT scans demonstrated a CR in all sites of disease. Response lasted 12 months at which time relapse was documented in the uterus. Additional Patients: two additional patients were treated with gemcitabine immediately following SGN-40, both achieved a PR.

Conclusions: Although these clinical observations were made outside of a controlled trial, the dramatic responses observed in these heavily pretreated patients suggest that SGN-40 may sensitize tumor cells to chemotherapy. These observations warrant further evaluation in clinical trials of SGN-40 in combination with gemcitabine and/or other chemotherapy regimens for the treatment of patients with NHL.

404 MORPHOIMMUNOHISTOCHEMICAL AND CLINICAL CHARACTERISTICS OF FOLLICULAR LYMPHOMA GRADE II

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Introduction: Approaches to treatment follicular lymphoma grade III (FL III) represent a difficult clinical problem. Now call in question validity of inclusion FL III in group of an intermediate grade.

Material and methods: FL III is established at 20 pts (22-78, median - 46 years) with using morphological and immunohistochecmical methods.

Results: At morphological research the heterogeneous picture is revealed. So, FL IIIA consists of patterns FL II in 35% of cases. Combining FL IIIB and DLBCL is established in 5% cases. Pts is positive in 75% of cases (threshold of an estimation >10% of positive cells). The high level of proliferative activity (Ki-67 >50%) is demonstrated in 5% cases. P53 is positive in 75% of cases (threshold of an estimation >10% of positive cells). The involvement of bone marrow is observed at 28% of pts. 21% pts are referred to group of intermediate risk, the group of high risk - 57% of pts.

Conclusions: Further study to confirm the possibility of using FL III in group of an intermediate grade.

405 AGE RELATED EPSTEIN BARR VIRUS ASSOCIATED B-CELL LYMPHOPROLIFERATIVE DISORDER: REPORT OF NINE PERUVIAN CASES

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Background: Age related Epstein Barr virus associated lymphoproliferative disorder is a new entity with aggressive course and poor survival. Oyama et al. described this new entity in 96 Japanese patients.

Patients and Methods: Nine Peruvian patients, diagnosed as having Age related EBV associated B cell Lymphoproliferative disorder were included in the report. All patients were positive for EBER using the in situ hybridization (ISH). Immunohistochemical expression of CD20, BCL6, CD10, and MUM-1/IRF4 was examined using a tissue microarray (TMA) technique.

Results: Mean age was 70 years old (range 31-85). Only one patient had less than 65 years old. Male/female ratio was 5/4. B symptoms occurred in 5/9. Zubrod >1 in 8/9 patients. Advanced stage (III/IV) in 8/9 patients. IPI more than 2 in 8/9 patients. Extranodal involvement was pleura (n=2), suprarenal gland (n=1), stomach (n=1) and bone marrow (n=1). Morphology in all cases was large cell lymphoma. All patients had a non germinal center like phenotype. Four patients died before treatment and five received CHOP regime. Two had complete response and three had progression disease. All cases died during first year and only one patient had a long survival (42 months).

Conclusions: Age related EBV associated B cell Lymphoproliferative disorder is a new entity, related to more advanced age, poor status performance, non-germinall center like phenotype and very short overall survival.

406 DETECTION OF DIFFERENT CLONAL EBV STRAINS IN HODGKIN LYMPHOMA AND NASOPHARYNGEAL CARCINOMA TISSUES FROM THE SAME PATIENT

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Introduction: The ubiquitous herpesvirus Epstein-Barr virus is linked to the development of several malignancies, including nasopharyngeal carcinoma (UCNT) and Hodgkin lymphoma (HL). However, the underlying mechanism for EBV-related malignancies has not been fully elucidated.

Material and Methods: In this study, we report the case of a patient with sequential development of UCNT and HL.

Results: A 29 year-old man was referred for nasal obstruction and cervical lymphadenopathy. Histologic examination of a lymph node concluded to nodal involvement by a nasopharyngeal carcinoma of the UCNT type. Tumour cells expressed the LMP-1 protein. The patient was treated by three courses of chemotherapy, followed by radiotherapy on nasopharyngeal and cervical areas. Evaluation after the therapeutic procedure concluded to a complete remission. Eight years later, the patient presented a centimetric mental adenopathy. Histologic examination of this adenopathy showed a HL of mixed cellularity type. Reed-Sternberg cells expressed the LMP-1 protein. The patient was treated by four courses of modified ABVD chemotherapy without radiotherapy. TEP scan after therapy showed a complete response. After a follow-up of 30 months, the patient is still in complete remission. DNA was extracted from the paraffin blocks of both tumours and was subjected to PCR protocols for detecting LMP-1 gene polymorphisms. We noted that HL samples had no 3 bp deletion whereas the UCNT sample was infected by a strain with a 30 bp deletion in its LMP-1 gene. These results clearly showed that the two tumours were infected by different viral strains.

Conclusions: The present report, which describes sequential development of HL and UCNT in one patient, seems exceptional. Our observation confirms the possibility of multiple EBV infections in a same individual. The two tumours contained different clonal viral genomes, suggesting a central and specific role of EBV strain infection in their pathogenesis.

407 BOVINE LEUKEMIA VIRUS AND HUMAN DETECTION OF ANTIBODY AGAINST BOVINE LEUKEMIA VIRUS

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Introduction: Bovine Leukemia Virus (BLV) is an oncogenic retrovirus that causes B cell leukemia in 1-5% of infected cattle. Most infected cattle do not actually develop leukemia but remain healthy and are not removed from the herd. BLV-infected cells are present in marketed beef and dairy products and consumption of unpasteurized dairy products or undercooked beef could possibly allow transmission of infectious virus to humans. BLV infection is not limited to cattle; the virus can infect sheep and nonhuman primates experimentally and cause cancer in the sheep in the laboratory and it can infect the cells of man species including humans and other primates. Researchers at the University of California, Berkeley found that a significant proportion of the American public may be harboring antibodies to Bovine Leukemia Virus, which they may have been exposed to through the consumption of beef or dairy products. Researchers found a disturbing trend. They found that geographically, the areas of highest cattle BLV infection did indeed seem to have significantly higher human leukemia rates.

Material and methods: This study was designed to see if any humans with acute lymphocytic leukemia (ALL) at all had antibodies to BLV. We propose here a pilot study to examine the first aspect of the overall proposal, whether humans can become infected with BLV. In this study, serum samples of fifty humans with ALL were examined for antibodies to BLV by commercial ELISA test and also for anti gp51 antibodies by AGID test.

Results: We didn’t find antibodies reactive specifically against BLV in people studied with ELISA test but one case showed precipitation line in AGID test.

Conclusions: Since AGID test is not a specific test, this study failed to reveal a strict relationship between BLV and human. However, we suggest that further studies in this area could be important, by the use of more sophisticated methods.

Key words: Bovine viral leukosis, Human, Cow.